On the effect of sampling rate and experimental noise in the discrimination between microbial growth models in the suboptimal temperature range.

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Abstract

Biochemical and microbial processes benefit from mathematical models. Often microbial kinetics are described as a function of environmental conditions in models exploited in predictive microbiology. Based on the organism different model structures are available. However, the aim is to determine the model that describes the system best.

This work deals with secondary models describing microbial kinetics in the suboptimal temperature range and their possibility to be discriminated. The used models are the cardinal temperature model with inflection and its adapted version. The method of Optimal Experiment Design for Model Discrimination is used to investigate the practical (in)feasibility of model discrimination given different noise and sampling frequency values.

Results point out the required steps and the possibilities of the method for model discrimination. It has been observed that discrimination is possible at various noise and sampling frequency levels. Moreover, also the corresponding increase in required experimental effort has been obtained.

Key words: predictive microbiology, model discrimination, dynamic modeling, optimization, optimal experiment design

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1. Introduction

Mathematical models are important tools for the analysis, monitoring, control and optimization of biochemical and microbial processes. Also for describing microbial processes in food and food processing mathematical models have been constructed (see, e.g., Saravacos and Kostaropoulos (1996); Blau et al. (2008)). These models have been typically used in view of product safety, product stabilization and process design and operation and are nowadays also extended towards food design (Trystram, 2012).

Microbial kinetics play a key role in these models as these kinetics determine the dynamic microbial evolution in time. The domain of *predictive microbiology* deals with mathematical models for describing these microbial kinetics as a function of environmental conditions. Depending on the microorganism and the application, different models are used for describing microbial growth, survival and/or inactivation in food products under possibly time varying environmental conditions. The environmental conditions can include, e.g., temperature, pH or background flora. A two step modelling approach is classically used in predictive microbiology. The first step consists of a *primary model*. This model describes the evolution of microbial concentration with time, under constant environmental conditions. In the second step, the parameters of the primary model are described by a *secondary model* as a function of changing environmental conditions (Baranyi and Roberts, 2004). When combining both primary and secondary models, microbial behavior can be described in a dynamic environment.

However, challenges when modeling microbial processes typically involve (i) the difficulty for performing experiments and obtaining numerous reliable data, (ii) the uncertainties on measurements and experimental data and (iii) the uncertainties concerning food properties Trystram (2012). Nevertheless, despite these challenges models which have a significant predictive power are desired. In this respect, first an appropriate kinetic model structure has to be found. In chemical engineering literature, this question has been widely studied and strategies for optimal design of (dynamic) experiments in view of model discrimination, i.e., *optimal experiment design for model discrimination* (OED/MD), have been reported , e.g., Burke et al. (1994); Buzzi Ferraris et al. (1984); Buzzi-Ferraris et al. (1990); Ungarala and Co (2000); Asprey and Macchietto (2000); Chen and Asprey (2003(@); Schwaab et al. (2008);

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Donckels et al. (2009, 2010); Luo et al. (2015).

There exist several criteria for discrimination. One of the first simple criteria has been developed by Hunter and Reiner (1965). For discriminating between two rival models, the new experimental condition should give model responses with the maximum difference. Since the first proposal, other criteria have been developed, with the criterion of Buzzi-Ferraris and Forzatti (1983) introducing the model deviations variance in the criterion. One of the latest extensions is the criterion proposed by Schwaab et al. (2008) and in parallel by Donckels et al. (2009). In this technique, the posterior covariance matrix of the difference between model predictions is taken into account during the design. Through this approach, apart from discrimination, also improved parameter estimates are achieved.

The aim of the current paper is evaluate the influence of practical limitations (e.g., limited sampling rate and inherent experimental noise) on the (im)possibility to discriminate between microbial kinetic models for growth in the suboptimal temperature range. This suboptimal temperature range (i.e., below the temperature at which growth is at its maximum) is of high importance for practical applications as this is typically in which food products are stored and transported. However, it is also a difficult temperature range to model as growth is typically slow, microbial concentrations are low and the experimental noise is high (compared to the low microbial concentration values).

Two well-known secondary models for microbial kinetics are selected. These models describe the influence of temperature on microbial growth: the cardinal temperature model with inflection (CTMI) (Rosso et al., 1993) and its adapted version (aCTMI). Up until now, exceptions have been reported only for *Listeria monocytogenes* (Bajard et al., 1996) and *Listeria innocua* (Le Marc et al., 2002). The difference for *Listeria* is in the suboptimal temperature region, where the plot of the square root of the maximum growth rate (μ_{max}) as a function of temperature, displays two linear phases. Listeria is the mircoorganism causing infections mainly to the central nervous system, i.e. listeriosis (Baron, 1996). The growth monitoring in the suboptimal temperature range is important for chilled, prepared food products (Le Marc et al., 2002).

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The paper is divided as follows. In Section 2 the mathematical models for describing the suboptimal temperature range are presented. Following in Section 3 the procedure for optimal experiment design for model discrimination is explained. Whereas in Section 4 the practical implementation is outlined. The results found are described and discussed in Section 5 and finally the main conclusions are summarized in Section 6.

2. Mathematical models for describing the suboptimal temperature range

As mentioned in the introduction, combining a primary with a secondary model allows to describe the microbial behavior in a dynamic environment.

The primary model used is the one proposed by Baranyi and Roberts (1994). The cell density is described as a function of time as seen below:

$$\begin{aligned}
&\frac{dn(t)}{dt} = \frac{Q(t)}{Q(t)+1} \cdot \mu_{max}(T(t)) \cdot [1 - \exp(n(t) - n_{max})] \\
&\frac{dQ(t)}{dt} = \mu_{max}(T(t)) \cdot Q(t) \\
&n(0) = n_0 \\
&Q(0) = Q_0
\end{aligned} \tag{1}$$

with $n(t)$ [ln(CFU/mL)] the cell density at time t [h], n_{max} [ln(CFU/mL)] the maximum value for $n(t)$ and μ_{max} [1/h] the maximum specific growth rate. $Q(t)$ is a measure for a physiological state of the cells. The initial values for $n(t)$ and $Q(t)$ for time $t = 0$ are n_0 and Q_0 respectively. In this work, $Q(t)$ is excluded, in other words it is assumed that there is no lag phase (see Van Derlinden et al. (2010) for details), and thus the model is reduced to:

$$\frac{dn(t)}{dt} = \mu_{max}(T(t)) \cdot [1 - \exp(n(t) - n_{max})] \tag{2}$$

The microbial growth rate as a function of temperature (secondary model) can be described by the CTMI (Rosso et al., 1993) and the aCTMI (Le Marc et al., 2002). For simplicity the temperature evolution $T(t)$ will be noted as T in the following.

The CTMI is described by:

$$\mu_{max}(T) = \gamma(T) \cdot \mu_{opt} \tag{3}$$

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with:

$$\gamma(T) = \begin{cases} 0 & T \leq T_{min} \text{ or } T \geq T_{max} \\ \dfrac{(T - T_{min})^2 (T - T_{max})}{(T_{opt} - T_{min})((T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T))} & T_{min} < T < T_{max} \end{cases} \tag{4}$$

The parameters included in this model are the three cardinal temperatures $T_{min}[^oC]$, $T_{opt}[^oC]$ and $T_{max}[^oC]$ (i.e., the minimum, optimum and maximum temperature for growth, respectively) and $\mu_{opt}[1/h]$ (the maximum specific growth rate at T_{opt}).

The aCTMI is described in a similar way as the CTMI but with a different $\gamma(T)$ function:

$$\gamma(T) = \begin{cases} 0 & T \leq T_{min} \text{ or } T \geq T_{max} \\ \dfrac{(T_c - T_1)^2 (T_c - T_{max})}{(T_{opt} - T_1)((T_{opt} - T_1)(T_c - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_1 - 2T_c))} \left(\dfrac{T - T_{min}}{T_c - T_{min}} \right)^2 & T_{min} < T \leq Tc \\ \dfrac{(T - T_1)^2 (T - T_{max})}{(T_{opt} - T_1)((T_{opt} - T_1)(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_1 - 2T))} & T_c < T < T_{max} \end{cases} \tag{5}$$

Apart from the previous four parameters the adapted model is defined also by $T_c[^oC]$, the so-called change temperature, and $T_1[^oC]$, the intersection point between the first linear part and the temperature axis. In Figure 1, the square root of the maximum growth rate as a function of temperature is displayed for the two models, and their difference in the region of T_{min} can be seen.

3. Procedure for optimal experiment design for model discrimination

When having to choose among two (or more) models, optimal experiment design for model discrimination is a reliable tool (see modeling cycle (Ljung, 1999)). In this section, the technique proposed by Schwaab et al. (2008) and Donckels et al. (2009), will be highlighted.

Optimal experiment design for model discrimination is a sequential procedure with several steps (see Figure 2). Initially, a preliminary experiment is required for an initial estimate of the model parameters. This experiment can either be available or specifically designed. When experimental data are

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available through an experiment, the model parameters are estimated in the parameter estimation task. When multiple models are available a model adequacy test can be performed in order to select the most suitable model. As a next step, if none of the models can be selected, a discrimination experiment has to be designed. The new designed experiment can be performed and subsequently, the model parameters can be re-estimated and the loop can continue until a model is selected.

In the current work the method is extended to dynamic models with varying input profile. The experimental degrees of freedom of the input are not restricted in time as in Donckels et al. (2009). The experimental design leads to a input profile varying over time that can be optimized. The measurement times are although fixed for every experiment as to decrease the optimized variables that make the problem already complicated.

In the following subsections the different steps-blocks of the procedure will be outlined, i.e. preliminary experiment, parameter estimation, model adequacy test and optimal experiment design for model discrimination.

3.1. Preliminary experiment

Initial values for the unknown model parameters are required for proceeding to model discrimination. These can result either from an arbitrary experiment or a designed one. The model parameters influence the discrimination procedure, therefore it is relevant to have an informative preliminary experiment. This can be attained through optimal experiment design for parameter estimation (Walter and Pronzato, 1997). In this method, the experiment is designed in such a way that the information content of the experiment is maximized with regard to the parameter estimates. The information content is quantified by the Fisher information matrix (FIM) (Walter and Pronzato, 1997).

$$\mathbf{F}(\mathbf{p}) = \sum_{i=1}^{N} \left(\frac{\partial \mathbf{y}(\mathbf{p},t_i)}{\partial \mathbf{p}}\right)^T \mathbf{Q} \left(\frac{\partial \mathbf{y}(\mathbf{p},t_i)}{\partial \mathbf{p}}\right) \tag{6}$$

with N the number of measurement at times t_i. $\mathbf{F}$ combines information on (i) the error on the output measurements ($\mathbf{Q}$ is typically defined as the inverse of the measurement error variance matrix), and (ii) the sensitivities

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of the model output ($\mathbf{y}(\mathbf{p}, t_i)$) to small variations in the model parameters $\mathbf{p}$ (expressed in the sensitivity matrix $\frac{\partial \mathbf{y}(\mathbf{p}, t_i)}{\partial \mathbf{p}}$).

The design criterion uses a scalar function of the FIM. The E-criterion, that maximizes the minimum eigenvalue of the FIM is a criterion that can be used among others (Walter and Pronzato, 1997).

3.1.1. Parameter estimation

One important underlying task is parameter estimation. Parameters as mentioned are coupled with the discrimination procedure. They need to be estimated before and after discrimination experiments. This can be done when experimental data and the matching inputs are available. Firstly, the parameters should be assumed identifiable (Jacquez and Greif, 1985; Chou and Voit, 2009). A next often made assumption includes that the probability distribution of the measurement errors is additive, independent and identically distributed according to a Gaussian distribution. When these assumptions hold parameters are selected such that the model predictions of $\mathbf{y}(\mathbf{p}, t_i)$ fit the observations $\mathbf{y}_i$, at times t_i, as accurately as possible despite the presence of measurement errors. Typically, the above settings lead to a *Weighted Sum of Squared Errors* objective (*WSSE*) (Walter and Pronzato, 1997):

$$WSSE(\mathbf{p}) = \sum_{i=1}^{N} (\mathbf{y}(\mathbf{p}, t_i) - \mathbf{y}_i)^T \ \mathbf{Q} \ (\mathbf{y}(\mathbf{p}, t_i) - \mathbf{y}_i) \tag{7}$$

3.1.2. Model adequacy test

Another important step for model discrimination is model selection. A statistical test can help indicating the outstanding model between the available ones.

When working in a in silico environment a statistical test that can be used is the χ^2-test. This test can prove a lack of fit (Chen and Asprey, 2003(@), and therefore the model failing this test can be excluded.

3.1.3. Optimal experiment design for model discrimination

The main task of discrimination between two models will be outlined in this subsection. The basis in model discrimination is to maximize a functional of the difference between the model predictions. The objective function

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J will maximize this functional. In the below presented method the posterior covariance matrix of the parameter estimates is included. Allowing apart from the increase of the discrimination power, which is the primary objective, also a decrease of the parameter variances (Schwaab et al., 2008; Donckels et al., 2009).

For discriminating between model $\mathcal{M}_1$ and $\mathcal{M}_2$, for experiment ω_{Ne+1} defined by input profile $u_{N_e+1}(\cdot)$ and time points t_i (with N_e the number of available experiments either preliminary or discrimination experiments), the discrimination function is defined at every t_i by:

$$\mathbf{D}_{1,2}(\omega_{N_e+1}) = d_{1,2}^T(\omega_{N_e+1})\mathbf{V}_{1,2}^{-1}(\omega_{N_e+1})d_{1,2}(\omega_{N_e+1}) \tag{8}$$

with:

$$\begin{aligned}
d_{1,2}(\omega_{N_e+1}) &= \mathbf{y_1}(\omega_{N_e+1}, \mathbf{p_1}) - \mathbf{y_2}(\omega_{N_e+1}, \mathbf{p_2}) \\
\mathbf{V}_{1,2}(\omega_{N_e+1}) &= 2\mathbf{V} + \mathbf{V}_1(\omega_{N_e+1}) + \mathbf{V}_2(\omega_{N_e+1}) \\
\mathbf{V}_1(\omega_{N_e+1}) &= \mathbf{B}_1(\omega_{N_e+1})\mathbf{V}_{p_1}(\omega_{N_e+1})\mathbf{B}_1^T(\omega_{N_e+1}) \\
\mathbf{V}_{p_1}(\omega_{N_e+1}) &= [\mathbf{B}_1^T(\omega_{N_e+1})\mathbf{V}^{-1}\mathbf{B}_1(\omega_{N_e+1}) + \mathbf{V}_{p,1}^{-1}(\omega_{N_e})]^{-1}
\end{aligned}$$

In the following formulas t_i is omitted for sake of simplicity. Here, $y_1(\omega_{N_e+1}, p_1)$ is the *prediction for model* $\mathcal{M}_1$ (similarly for model $\mathcal{M}_2$), $\mathbf{V}_{1,2}(\omega_{N_e+1})$ is the *posterior covariance matrix* of the differences between model predictions, K is the number of discrete time points t_k, $\mathbf{V}$ is the *covariance matrix of the experimental deviations* and $\mathbf{V}_1(\omega_{N_e+1})$ is the *covariance matrix of model prediction variations* calculated for model $\mathcal{M}_1$ (and similar for model $\mathcal{M}_2$). The model uncertainty includes the uncertainty on the model predictions and on the measurements (Schwaab et al., 2008; Donckels et al., 2009).

$\mathbf{B}_1(\omega_{N_e+1})$ is the *sensitivity matrix* that contains the first derivatives of the responses of model $\mathcal{M}_1$ with respect to its parameters $\left(\dfrac{\partial \mathbf{y_1}(\omega_{N_e+1}, \mathbf{p_1})}{\partial \mathbf{p_1}}\right)$.

$\mathbf{V}_{p_1}(\omega_{N_e+1})$ is the *posterior covariance matrix of model parameters*. It can be seen that $\mathbf{V}_{p_1}$ consists two parts, i.e., the covariance matrix of the new designed experiment with experiment condition $T_{N_e+1}(\cdot)$ and the current covariance matrix of the parameter estimates. The covariance matrix of the estimated parameters is approximated by the inverse of the Fisher information matrix (FIM), since the errors are assumed independent (Walter and Pronzato, 1997).

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4. Implementation

In the work of Le Marc et al. (2002), it has been experimentally shown that for *L. innocua*, the aCTMI can describe more accurately its behavior in the suboptimal temperature region. In other words, the CTMI and aCTMI differ for this micro organism.

Since the parameters for aCTMI are documented in Le Marc et al. (2002), it can be used as a simulation case study of the OED/MD method.

As mentioned in the introduction, the objective of this study is to evaluate whether the method of OED/MD is able to discriminate between the two models (CTMI and aCTMI) under different complexity factors. For this reason five different measurement noise levels have been tested along with five different sampling patterns. By this approach both theoretical as well as more realistic scenarios are tested.

4.1. Pseudo-measurements generation

The parameters from Le Marc et al. (2002) have been used for generating pseudo-measurements for aCTMI and can be seen in Table 1. Noise drawn from a Gaussian distribution with zero mean and variance σ^2 as seen in Table 2, has been added to the pseudo-measurements.

The value $\sigma = 0.1808$ is used in literature for these type of micro organisms (Van Derlinden et al., 2008). Another value traditionally used is the confidence bound of $1 \log 10$(CFU/ml) for every measurement (Jennison and Wadsworth, 1940). It is known that the the 95.44% confidence region is equal to 4 σ (Mood, 1974). After some transformations it can be found that the corresponding sigma value is $\sigma = 0.5874$. The other values have been chosen to expand the range evenly.

The two models differ at the suboptimal temperature region and therefore the discrimination will be focused in this region. Parameters T_{opt} and T_{max} have been kept constant and identical for both models with values 37.4 and 45.4^oC, respectively. One experiment is assumed to have a time horizon of 120 hours and a sampling rate of 4, 6, 8, 10 and 12 hours (five different cases). The initial concentration (n_{init}) is set at 7 ln(CFU/ml) and the maximum growth n_{max} is set at 22.55 ln(CFU/ml).

Table 1: Parameter values for Listeria for aCTMI as presented in Le Marc et al. (2002)

model	μ_{opt}[1/h]	T_{min}[^{o}C]	T_c[^{o}C]	T_1[^{o}C]
aCTMI	1.14	-4.5	10	0.6

4.2. Input profile

The temperature profile that has been optimized is parametrized as in Van Derlinden et al. (2010). For optimizing a temperature profile it is necessary to parametrize it in a mathematical way. For representing the profile of Figure 3 four degrees of freedom are needed. These are the initial temperature T_0 [^{o}C], the time at which the increase or decrease in temperature starts t_s [h], the rate of temperature change $\Delta T/\Delta t$ [^{o}C/h] and the duration of the temperature change Δt [h].

Table 2: Sigma values and sampling frequency used

σ [ln(CFU/ml)]	0.1808	0.4	0.5874	0.8	1
δt [h]	4	6	8	10	12

4.3. Discrimination procedure implementation

The first step is to design a preliminary experiment for getting an initial estimate of the parameters. This can be done by using optimal experiment design for parameter estimation and more specific the E-criterion for the aCTMI (Walter and Pronzato, 1997; Franceschini and Macchietto, 2008). The design is focused on aCTMI, as it has more unknown parameters.

After the first designed experiment for parameter estimation pseudo-measurements are created and the parameters of the two models are estimated based on these measurements. If the estimated parameters are not accurate enough a new experiment is designed and the parameters are re-estimated based on the two experiments. For the current work three subsequent experiments were designed for a sufficient estimate of the parameters.

The next step is to use OED/MD for designing experiments for discriminating between aCTMI and CTMI. The obtained model parameters from

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the preliminary experiment are used in the technique as explained in Section 3.1.3. The OED/MD procedure provides a new input profile, i.e. the optimization returns the four parameters T_0, t_s, $\Delta T/\Delta t$ and Δt. The discrimination power of an input is calculated through the objective function (Equation 8) for the entire experimental horizon at specified sampling points. In contrast to Donckels et al. (2009) where the discrimination power is at one specific sampling time evaluated, in this work the computational time and complexity are increased due to the fact that the entire input profile is varying over time.

Using the new obtained input profile a silico experiment is preformed, i.e., pseudo-measurements are created. The two models are re-fitted using the preliminary experiments together with the new experiment.

An additional discriminatory experiment is designed and the parameters are re-estimated based on all five experiments. In total up to 8 sequential discrimination experiments have been performed for this study.

4.4. Computer tools

The parameter estimation is performed with the `lsqnonlin` matlab function from the optimization toolbox. This function solves a least squares problem using the trust-region-reflective algorithm (Coleman and Li, 1996).

For the optimal experiment design for parameter estimation and model discrimination the maximization problem is solved with the `patternsearch` matlab function from the global optimization toolbox. This function finds the minimum of the objective function using a pattern search algorithm (Audet and Dennis Jr, 2003). For a better result `patternsearch` is combined with a multi-start approach with 10 and 20 different starting points.

5. Results and discussion

The aim of this paper is to thoroughly study and evaluate the method of model discrimination for microbial kinetics models. For this reason 25 different combinations of noise levels and sampling rates have been studied. Through these different combinations an overall view of the application and complication of this method will be achieved.

One single experiment in the laboratory would require the experiment time (here 36 hours) plus the preparation before and after. In total 4 days are required for one experiment. Therefore it is essential to study the different scenarios in silico and having a better insight for this method.

Discrimination is noted as achieved when the WSSE value of one model is higher than the corresponding χ^2-value. The evolution of the achieved discrimination among the experiments is displayed in Figure 4. The event of none of the two models CTMI and aCTMI can describe the experimental results is represented by a red circle (o). Whereas when one model is discarded, thus its WSSE value is higher than the χ^2-value, a green plus sign (+) is shown. For this case study when a model is discarded it is always the CTMI. It can be seen that there is a gradual increase in the amount of discrimination experiments needed as the complexity increases. For example for a sampling time of 4 hours and a $\sigma = 0.4$ only one discrimination experiment is required whereas for a sampling time of 10 hours and $\sigma = 1$ 6 discrimination experiments are needed.

For a closer view on the outputs a combination of experimental conditions has been selected for comparison. Choosing a middle point of graph 4 after one discrimination, i.e. $\sigma = 0.5874$ and sampling rate 8 hours, as basis for comparison with other points around, will give a nice view.

In Figure 5 the outputs of all performed experiments (top left top right and bottom left) and the corresponding temperature profiles (bottom right) are displayed for $\sigma = 0.5874$ and sampling rate 8 hours. The results are displayed after the preliminary experiments (top left), one discrimination experiment (top right) and two discrimination experiments (bottom left). It gives a nice overview of how no difference between the two models is seen after only the preliminary experiments. However, after one discrimination there is a slight difference which is increased after two discrimination experiments, where also the WSSE value (see Table 3) confirms the possible discrimination. It is important that all performed experiments are analysed since the estimated parameters and the discrimination value are calculated based on all available experiments. The discrimination experiment with the rather low but constant temperature focusses on making a difference in the low temperature zone when not many micro-organisms are present. This is also the region of interest. However, due to the slow growth a long time horizon

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Table 3: WSSE values and the corresponding χ^2 values for the compared cases. Discrimination is achieved when one model exhibits a WSSE value lower than the corresponding χ^2 value, while the other model exhibits a WSSE value higher than the corresponding χ^2 value. In this case the latter model can be excluded.

		discrimination	CTMI		aCTMI	
σ	sampling time [h]	experiments	WSSE	χ^2	WSSE	χ^2
0.5874	8	0	28.24	62.83	28.13	60.48
0.5874	8	1	70.16	81.38	44.80	79.08
0.5874	8	2	104.24	99.62	61.91	97.35
0.1808	8	1	164.49	148.78	115.08	146.57
1.0000	8	1	53.03	58.12	43.22	55.76
0.5874	4	1	294.36	81.38	58.72	79.08
0.5874	12	1	62.71	81.38	56.52	79.08
0.5874	8	6	277.15	170.80	143.29	168.61

has to be taken and the corresponding parameter accuracy may not be too high for all parameters. The discrimination experiment with the high initial temperature tries to elucidate the difference at much higher concentrations of micro-organisms. This last one has the advantage that differences will be visible earlier and that parameter accuracy will be higher. Nevertheless, combining both enables focussing on model discrimination (while improving also the parameter accuracy) in the low and high concentration zone at low temperatures.

As a next step to the comparison in Figure 6 the outputs after the first discrimination experiment (right) and the corresponding temperature profiles (left) can be seen for $\sigma = 0.1808$ (top), $\sigma = 0.5874$ (middle) and $\sigma = 1$ (bottom) and sampling rate 8 hours. In this case the influence of the noise complexity can be observed. For the low value $\sigma = 0.1808$ only after one discrimination experiment discrimination is visible whereas for $\sigma = 0.5874$ and even more for $\sigma = 1$ this is not the case. For $\sigma = 0.5874$ discrimination is achieved after one more experiment (2 discrimination experiments). Whereas for $\sigma = 0.5874$ two more experiments are needed (3 discrimination experiments in total). The corresponding WSSE values can be seen in Table 3).

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Another complexity factor, i.e., the sampling rate, can be studied in Figure 7. Where the outputs after the first discrimination experiment (right) and the corresponding temperature profiles (left) can be seen for $\sigma = 0.5874$ and sampling rate 4 hours (top), 8 hours (middle) and 12 hours (bottom). Again as before in the high sampling rate discrimination is possible whereas for the lower sampling rate it can not be seen as confirmed by the WSSE values (see Table 3). For the 8 and 12 hour sampling rate one more discrimination experiment is needed for achieving discrimination (in total 2 discrimination experiments). Every experiment when sampling at 4 hours has 31 samples, whereas for 8 and 12 hours the sampling points are 16 and 11, respectively. This means that for the 4 hours sampling 124 points are required for discrimination, whereas for the 8 and 12 hours 80 and 55 points (5 experiments in total). The experimenter should then weight the effort of more sampling points per experiment in contrast to more experiments but with less sampling.

Finally the outputs (right) and the corresponding temperature profiles (left) of the reference point with $\sigma = 0.5874$ and sampling rate 8 hours after six discrimination experiments is seen in Figure 8. Discrimination can not be overlooked and it can be concluded that the higher the number of experiments is the higher the discrimination power. The WSSE values that confirm the discrimination can be found in Table 3).

As expected, as the noise increases more discrimination experiments are required. The same holds for the sampling points the higher the distance between the measurement points the more experiments needed. This can be explained from the following factors: a) the parameter uncertainty increases as the noise increases and/or the sampling frequency decreases, b) the difference between the two models is less distinguishable as the noise increases and c) the less measurement points are available at every experiment the more experiments are needed for acquiring the same information.

6. Conclusions

In this work the method, the possibility to exploit Optimal Experiment Design for Model Discrimination in practice has been evaluated in silico for a two rival secondary models describing microbial kinetics. In practice, the quantity and the quality of measurements are limited in a microbial system. More specifically, different complexity cases have been studied including various noise and sampling frequency values. Through this study an overall view of the possibilities of this method is obtained and an indication of the required experimental burden has been found. As the conditions become more complex, more discrimination experiments are needed as expected. Although the parameters are more difficult to be estimated in the higher noise values, the method applied overcomes this difficulty achieving both discrimination and more accurate parameters.

Assuming the microbial behavior follows the adapted CTMI model (as is the case for the Listeria monocytongens) it can be concluded that it is possible to discriminate at all given the complexity that characterizes real-life application.

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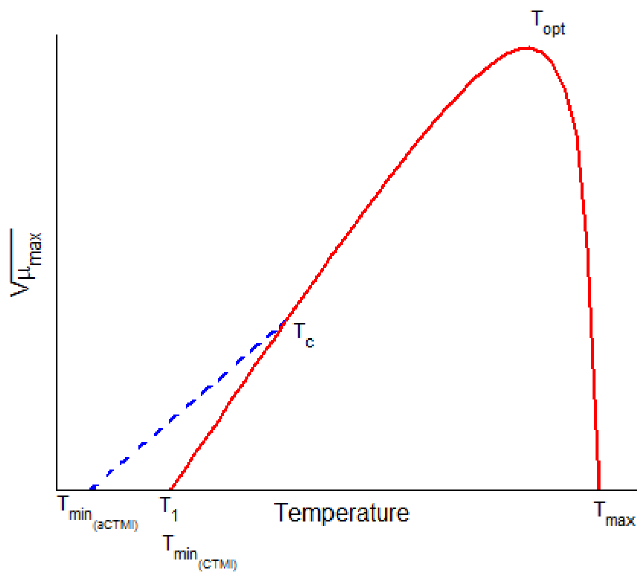

Figure 1: The maximum specific growth rate $\sqrt{\mu_{max}}$ as a function of temperature, as described by the CTMI (-) and aCTMI (- -) models.

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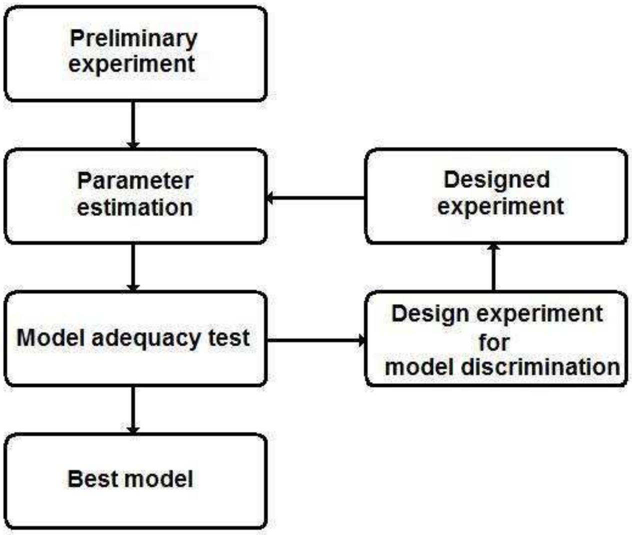

Figure 2: Steps for OED-MD (after Donckels et al. (2009))

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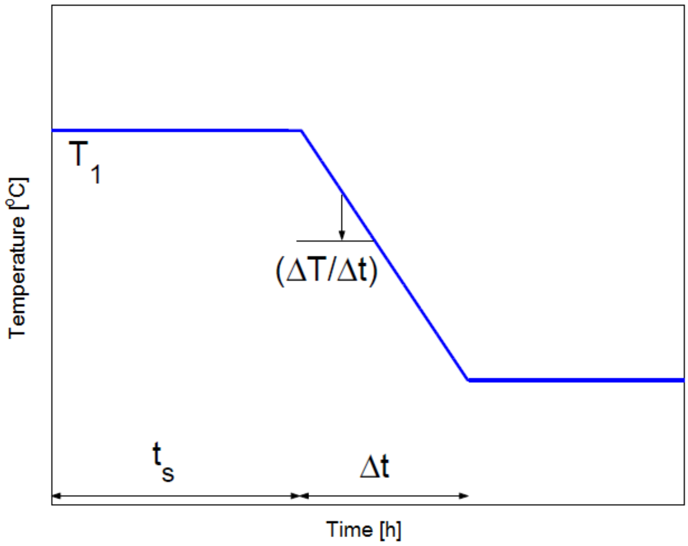

Figure 3: Parameterized temperature profile characterized by four degrees of freedom: T_1 [^{o}C] the initial temperature, t_s [h] the time at which the increase or decrease in temperature starts, $\Delta T/\Delta t$ [^{o}C/h] the rate of temperature change and Δt [h] the duration of the temperature change (Van Derlinden et al., 2010).

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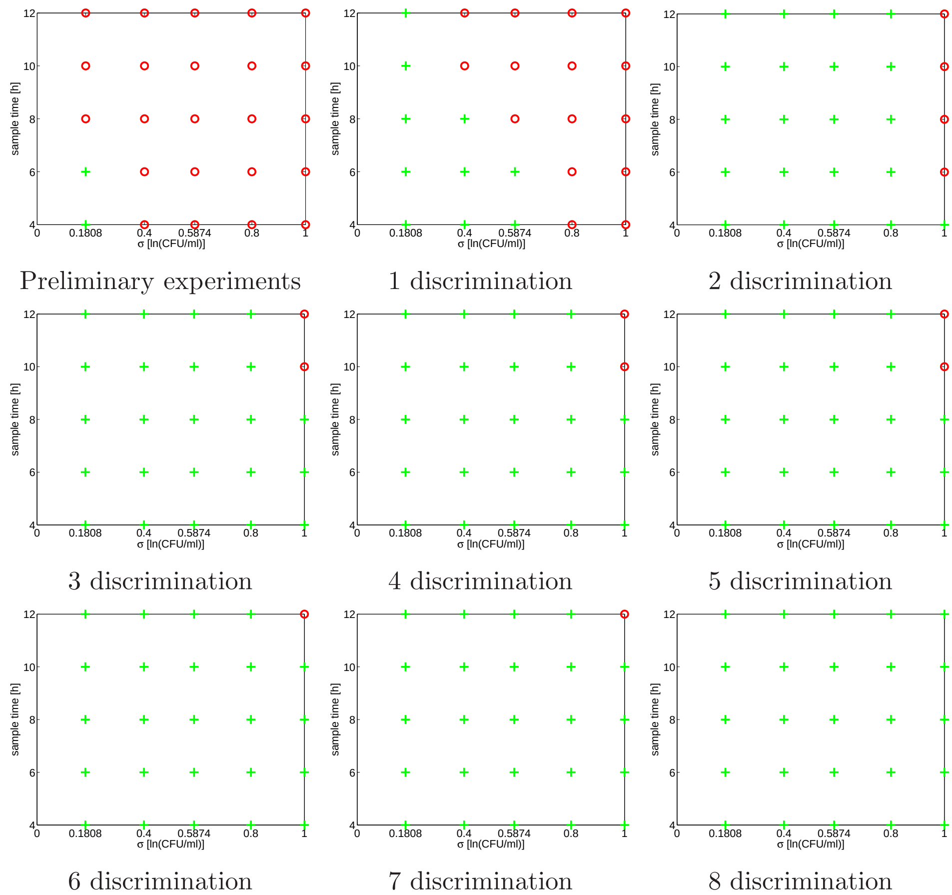

Figure 4: Evolution of achieved discrimination, green (+) achieved discrimination and red (o) otherwise.

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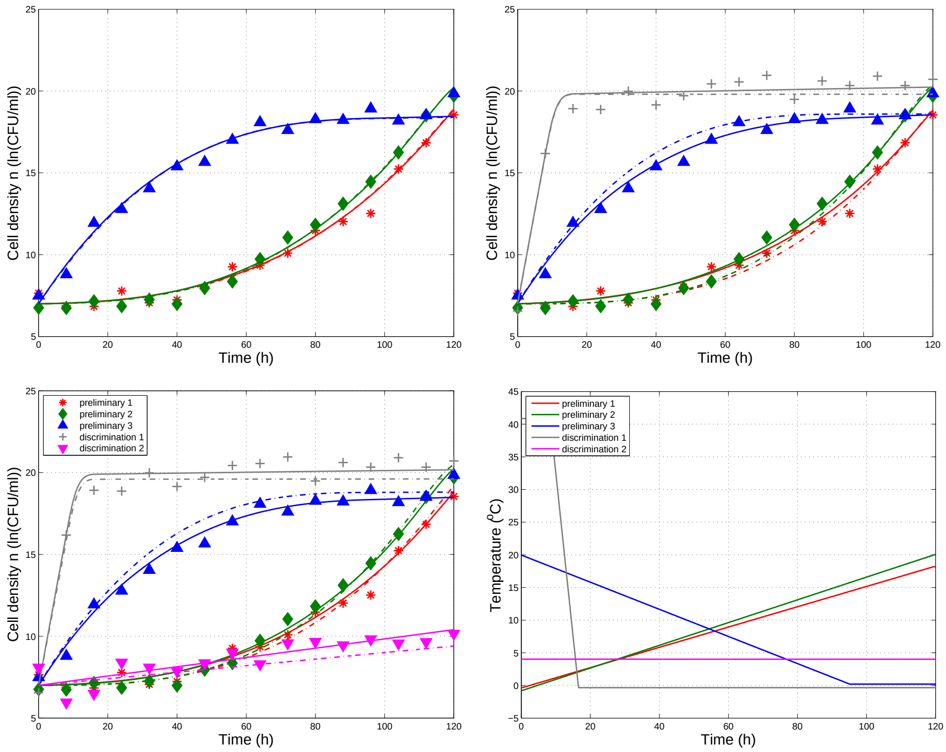

Figure 5: CTMI (- -) and aCTMI (-) model predictions together with pseudo-measurements after the three preliminary experiments (top left), after one discrimination experiment (top right) and after two discrimination experiments (bottom left), for $\sigma = 0.5874$ and sampling ratio 8 hours, and the corresponding temperature profiles (bottom right). It has to be noted that each time all available data sets are used to estimate a single set of CTMI / aCTMI parameters.

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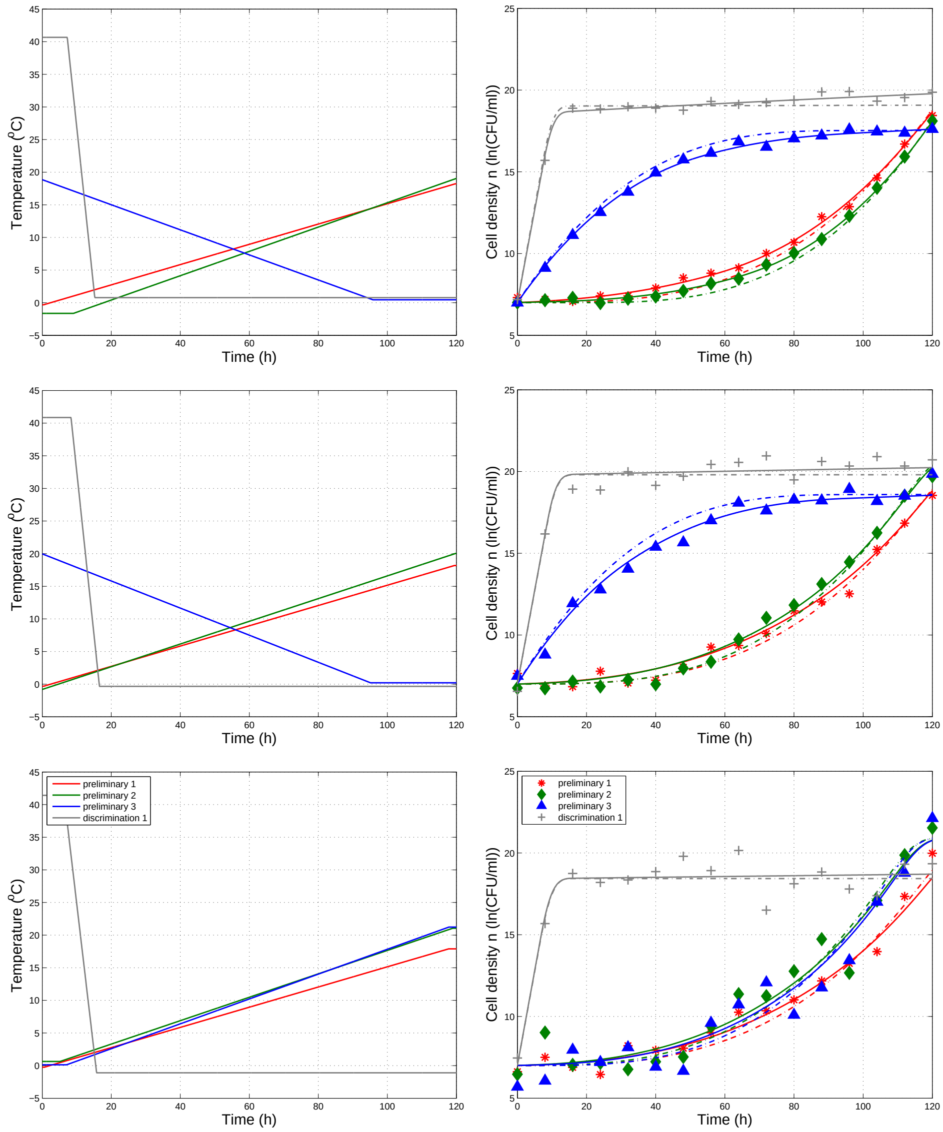

Figure 6: CTMI (- -) and aCTMI (-) model predictions together with pseudo-measurements (right) and corresponding temperature profiles (left) after one discrimination experiment for sampling ratio 8 hours and $\sigma = 0.1808$ (top), $\sigma = 0.5874$ (middle) and $\sigma = 1$ (bottom)

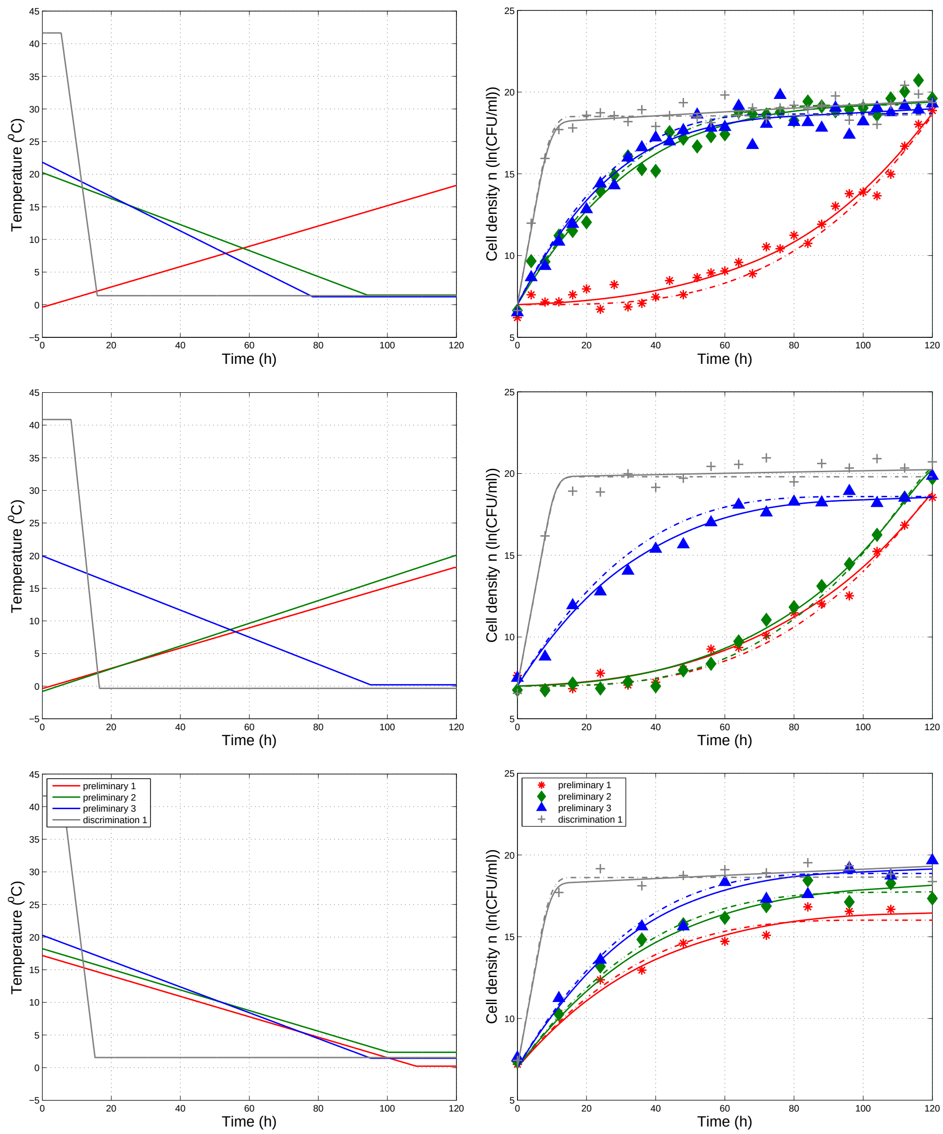

Figure 7: CTMI (- -) and aCTMI (-) model predictions together with pseudo-measurements (right) and corresponding temperature profiles (left) after one discrimination experiment for $\sigma = 0.5874$ and sampling ratio 4 hours (top), 8 hours (middle) and 12 hours (bottom)

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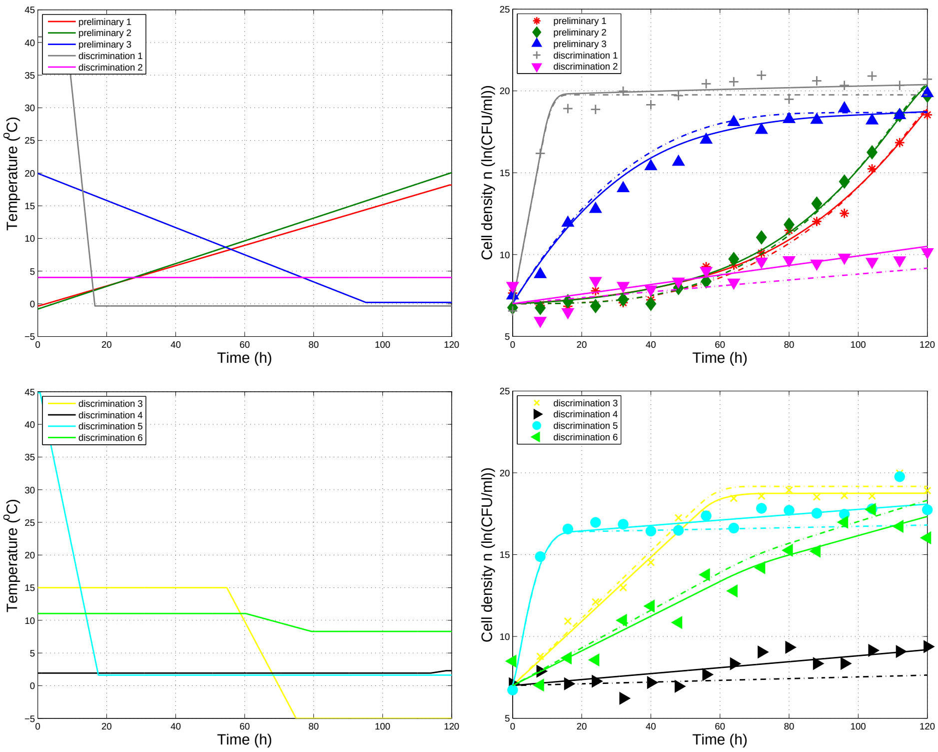

Figure 8: CTMI (- -) and aCTMI (-) model predictions together with pseudo-measurements (right) and the corresponding temperature profiles (left) after six discrimination experiments for sampling ratio 8 hours and $\sigma = 0.5874$

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